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DOES AN INCREASE IN DIETARY LINOLEIC ACID MODIFY TISSUE CONCENTRATIONS OF CERVONIC ACID AND CONSEQUENTLY ALTER ALPHA-LINOLENIC REQUIREMENTS? MINIMAL REQUIREMENT OF LINOLEIC ACID IN ADULT RATS.

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SUMMARY

Rats were fed a control diet containing both linoleic and alpha-linolenic acid. When 60-days-old they were divided into 8 groups, each receiving the same amount of alpha-linolenic acid, but varying amounts of linoleic acid. When the (n-6)/(n-3) ratio in the diet varied from 2 to 32 (with a constant amount of 150 mg alpha-linolenic acid per 100 g diet), tissue levels of the (n-3) series fatty acids were not significantly modified, except in the liver, heart and testes. In all organs studied, the saturated and monounsaturated fatty acids were practically unchanged. For the (n-6) series fatty acids, arachidonic acid was not significantly affected, in muscle, kidney, brain , myelin, nerve-endings or sciatic nerve, whatever the quantity of linoleic acid in the diet. In liver, arachidonic acid plateaued at 2400 mg linoleic acid/ 100 g diet and at 400 mg /100g diet in heart. Results for 22:5(n-6) showed a marked increase in heart, a moderate increase in liver and kidney, and no effect in muscle, testes, brain, myelin, nerve-endings or sciatic nerve. This experiment defined the minimum amount of linoleic acid required in the diet to maintain fatty acids of the linoleic family in the young adult rat. For the first time it was demonstrated that 1200 mg/100 g diet are sufficient for the liver, as evidenced by maintenance of the arachidonic acid concentration. For the other organs, there is either a very marked preservation of this acid, or the dietary level is less than 300 mg/100 g diet. For the essential fatty acid precursors (i.e. linoleic and alpha-linolenic acids), the optimal (n-6)/(n-3) ratio required in the diet is about 8.

INTRODUCTION

Linoleic acid is now universally recognized to be an essential nutrient. The relationship between the intake of linoleic acid and the arachidonic level in tissues and organs is of special nutritional interest. The polyunsaturated fatty acids found in membranes are derived from the dietary precursors (linoleic and alpha-linolenic acids), and they have longer and more highly unsaturated chains (mainly arachidonic and cervonic acids). These acids, in particular arachidonic acid, are the precursors of important hormonal substances. (prostaglandins and leukotrienes), and play important roles in the

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mechanism of action of second messengers, but their structural role is also important since they modulate the structure, enzymatic activities, and function of the membranes. The effect of diets containing different amounts of linoleic acid on the levels of polyunsaturated fatty acids in tissue lipids has been examined in experimental animals by many authors. These studies were designed to determine the changes in fatty acid profiles of tissue and plasma lipids. But the various diets used consisted of different types of fats containing markedly different proportions of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (of both n-6 and n-3 series). Moreover, changes in alpha-linolenic acid or even oleic acid can modify linoleic utilisation or metabolism.Thus, the direct relationship between changes in dietary linoleic acid and the accumulation of this acid and its derivatives in tissues has not been specifically investigated.

In one experiment, when linoleic intake was increased in one-month-old rats, there was an accumulation of arachidonic only in the liver. In other organs, such as heart, the major changes were a decline in highly unsaturated fatty acids of both the n-3 and n-6 series (1). In another experiment, the minimal dietary requirement of linoleic acid was determined for various rat organs during the gestational-lactating period by feeding the animals different amounts of linoleic acid, but a constant amount of alpha-linolenic acid (2).

It is now clear that polyunsaturated fatty acid deficiency alters the composition and structure of membranes in all types of cells, including those of the nervous system. The presence of alpha-linolenic acid, as well as various dietary, hormonal, or toxic factors, can affect linoleic acid metabolism (3,4). Essential fatty acid deficiency affects all nerve functions, including conductivity, and there are changes in the electroretinogram and in behavior (5,6). The effects of deficiency have also been described in man and can result from maternal or perinatal deficiency, undernutrition in the adult, or unsuitable enteral or parenteral feeding (7,8). Most experiments, mainly in the rat and mouse, performed with varying (n-6)/(n-3) ratios involved changing amounts of alpha-linolenic acid, and not of linoleic acid (9-12).

Over the last decades, several panels of experts have, in fact, recommended that linoleic acid intake be increased up to 7 to 10 percent of energy intake. This value is estimated to be above the minimum requirement. *In vitro* studies indicate a high rate of production of the long-chain, highly unsaturated fatty acids in liver (13-18). The conversion rate of linoleic acid into longer chains is not known, neither in a given organ nor in the whole organism.

The quantities of alpha-linolenic (18:3n-3) and linoleic (18:2n-6) acids required in the diet to build the membrane structures of various organs including brain have been determined (19). Recent experiments have permitted measurement of the quantities necessary to maintain these structures, i.e. to ensure their turnover. In view of the metabolic interrelations between the linoleic and alpha-linolenic families, in particular the inhibition of the use of one by the presence of an excess of the other, it is important to clarify to what extent a slight excess of dietary linoleic acid could modify the utilisation of alpha-linolenic acid. The objective of this work was to determine the linoleic acid requirement of the brain and other organs when the requirement in alpha-linolenic acid was concurrently satisfied. Requirements are defined as the minimal amount of linoleic acid that maintains a constant amount of 20:4n-6 in all tissues, and the minimal amount of dietary alpha-linolenic acid to obtain a constant amount of 22:6n-3 in all tissues (i.e 0.4 % of calories) (19). For this purpose, 60-day-old rats were divided into 8 groups. All of them received the same quantity of alpha-linolenic acid in their diet, but each group received different quantities of linoleic acid, with the (n-3)/(n-6) ratio ranging from 2 to 32. Fatty acid profiles were determined in different organs. The objective was more particularly to clarify whether high quantities of linoleic acid in the diet perturbed the quantity of cervonic (22:6n-3) and (22:5n-6) acids in the tissues. Two major indices were calculated: 22:6(n-3)/22:6(n-3)+22:5(n-6) and 22:5(n-6)/22:6(n-3)+22:5(n-6).

METHODS

Approximately 150 Wistar rats received a control diet containing 5% lipids as a mixture of peanut and rapeseed oil (50/50) supplemented with 3% hydrogenated palm oil up to the age of 60 days. This supplied, per 100 g diet, 1200 mg of 18:2(n-6) and 200 mg of 18:3n-3 (n-6/n-3 = 6). From the age of 60 days, the animals were divided into 8 groups. One continued to receive the control diet, the 7 others received one of the 7 diets each containing 8% lipids. These 7 diets all supplied 150 mg of 18:3(n-3)/100 g diet but increasing quantities of 18:2(n-6), such that the n-6/n-3 ratio ranged from 2 to 32 (2,4,8,12,16,24,32). Animals were sacrificed 4 weeks later. The quantities of oils used and the diet compositions are given in tables 1 and 2. Animals were sacrificed 2 months after the change in diet and fatty acid profiles were determined for the following: whole brain, brain organelles (myelin, synaptosomes), sciatic nerve, and various organs (liver, kidney, testes, heart, muscle). Lipids were extracted with chloroform-methanol according to Folch; methyl esters were obtained according to Morrisson with boron fluoride-methanol; and fatty acids were analysed by gas chromatography using a capillary column. The techniques developed in our laboratory have been published (19).

Statistical significance of mean differences between dietary groups was tested by analysis of variance (two way ANOVA , alpha = 0.05). For the various organs, values in the figures are the mean of at least five different animals, from at least three different litters. For myelin and nerve endings, each value is the mean of at least four different

Table 1 Composition of oils used

Oil	Levels 18:2(n-6) (wt %)	18:3(n-3) (wt %)
Sunflower	66.5	trace
African peanut	19.6	trace
Hydrogenated palm	2.0	-
Soybean	55.0	6.8
Rapeseed	21.7	7.0
Linseed	15.1	56.1

Table 2 Composition of diets

DIETS	Controls	1	2	3	4	5	6	7
OILS (mg)								
Rapeseed	2875	-	2156	2156	2156	2156	2156	-
Peanut	3132	695	304	3929	-	-	-	-
Linseed	-	285	-	-	-	-	-	-
Sunflower	-	-	-	-	2063	3053	5032	5479
Soybean	-	-	-	-	-	-	-	2660
Hydrogenated palm	1993	7020	5540	1915	3781	2791	812	-
(n-3)	200	150	150	150	150	150	150	150
(n-6)	1200	300	600	1200	1800	2400	3600	4800
(n-6)/(n-3)	6	2	4	8	12	16	24	32

preparations; each density gradient required at least four animals. Thus, each value represents at least 16 animals (from at least three different litters). Experimental protocols were approved and met government guidelines (Ministery of Agriculture, authorization n° 03007, June 4, 1991).

RESULTS AND DISCUSSION

Regardless of the n-6/n-3 ratio, the saturated and monounsaturated fatty acid concentrations of the different organs were practically unchanged (data not shown).

On the other hand, the polyunsaturated fatty acids showed considerable variations in some organs. The amount of arachidonic acid (Figure 1) was very little altered in

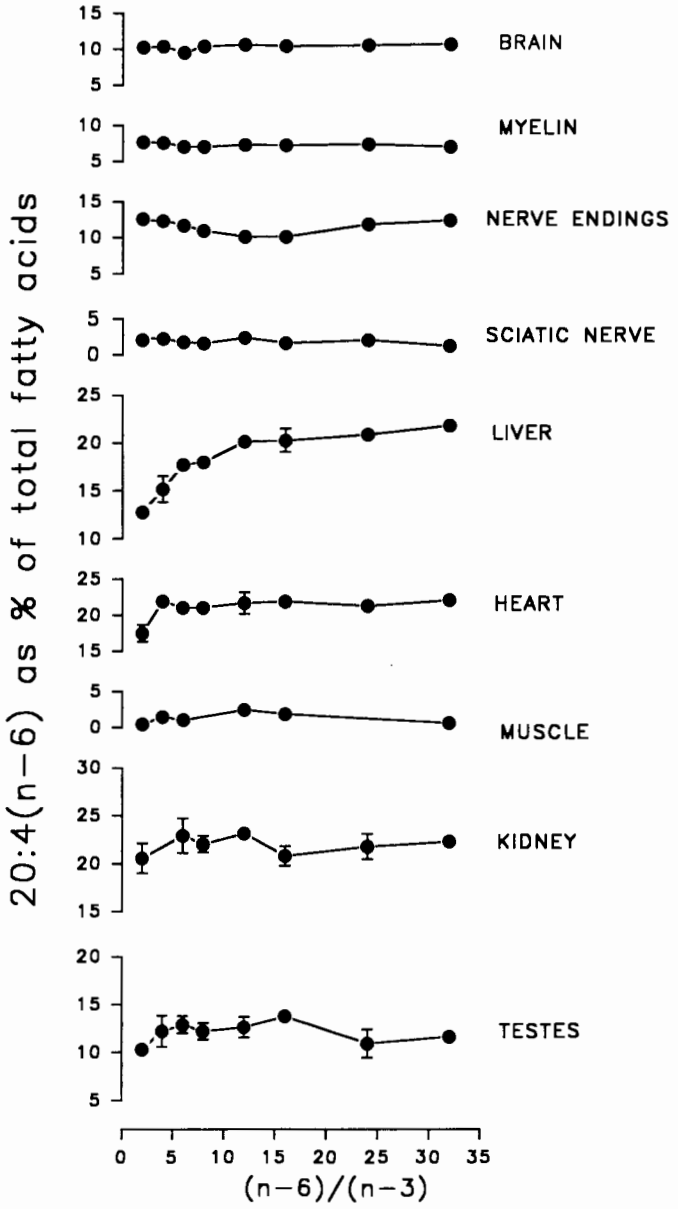

Figure 1. Amount of arachidonic acid as a function of dietary (n-6)/(n-3) ratio in different tissues.

certain organs by a decrease in dietary linoleic acid. In contrast, in liver the level fell if the linoleic acid content was less than 2400 mg/100 g diet, i.e. a (n-6)/(n-3) ratio less than 8. The two indices enable the results to be summarized as follows:

The first index (Figure 2) shows the variations in the 22:6(n-3)/22:(n-3) + 22:5(n-6) ratio. In brain, liver, kidney, and sciatic nerve this index remains constant whatever the (n-6)/(n-3) ratio in the diet. In heart, it decreases slightly with the increase in the ratio. In testes, the index decreases markedly. It should be noted that this index is close to 1 in organs in which it does not vary except in heart. It is lower than 0.1 in testes which show marked variations.

The second index (Figure 3) shows the variations in the 22:5(n-6)/22:6(n-3) + 22:5(n-6) ratio. This index remains constant only in testes, sciatic nerve, and muscle. It increases regularly with increase in the (n-6)/(n-3) ratio in kidney, liver, and heart. In the brain, it increases slightly up to a ratio of 6, and then stabilizes.

For fatty acids of the (n-6) family, these results show that:

(i) In the liver, the minimum dietary requirement of linoleic acid to maintain the levels of the (n-6) fatty acids is measurable. The minimum ratio must be 8, i.e. a minimum intake of 1200 mg linoleic for 150 mg alpha-linolenic acids per 100 g diet. For the other organs it was not possible, under the conditions of our experiment, to specify the minimum intake of linoleic acid required to maintain levels of (n-6) series fatty acids. This result shows that either arachidonic acid is very well preserved, or that a dietary intake less than 300 mg linoleic acid per 100 g diet is sufficient.

(ii) It was not possible to specify the maximum linoleic acid intake since an increase in its intake or in the (n-6)/(n-3) ratio does not result in accumulation of arachidonic acid.

For fatty acids of the (n-3) family, results show that:

(i) Except in brain, the (n-6)/(n-3) ratio as well as the quantity of linoleic acid in the diet is either without effect on the two indices, or one index increases and the other decreases regularly and linearly to a different degree depending on the organ.

(ii) For brain, the (n-6)/n-3) ratio and the amount of linoleic acid in the diet have strictly no effect on the 22:6(n-3)/22:6(n-3) + 22:5(n-6) ratio. On the other hand, interpretation of the 22:5(n-6)/22:6(n-3) + 22:5(n-6) ratio is more difficult. In fact, supposing that 22:5(n-6) merely replaces 22:6(n-3) when the latter is deficient, it can be considered that 22:5 (n-6) should stabilize at its lowest level. In this case, the optimal (n-6)/n-3) ratio would be 4. However, at levels higher than 6, this ratio remains stable, whatever its value. This result seems to suggest that 22:5(n-6) could play another role than that of more replacement; in this case, the minimum (n-6)/n-3) ratio required in the diet would be about 6. However, these conclusions are based on small but significant variations.($p < 0.05$) and on a very small index (less than 0.1).

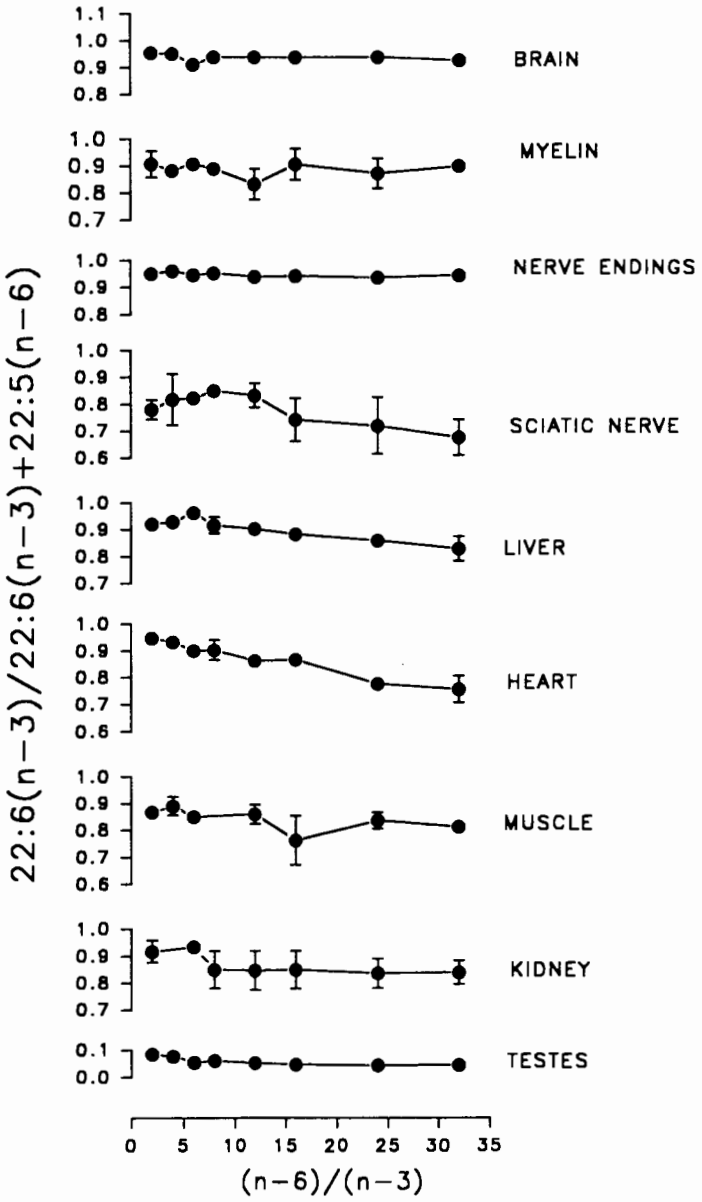

Figure 2. Ratio 22:6(n-3)/22:6(n-3)+22:5(n-6) as a function of dietary (n-6)/(n-3) ratio in various tissues.

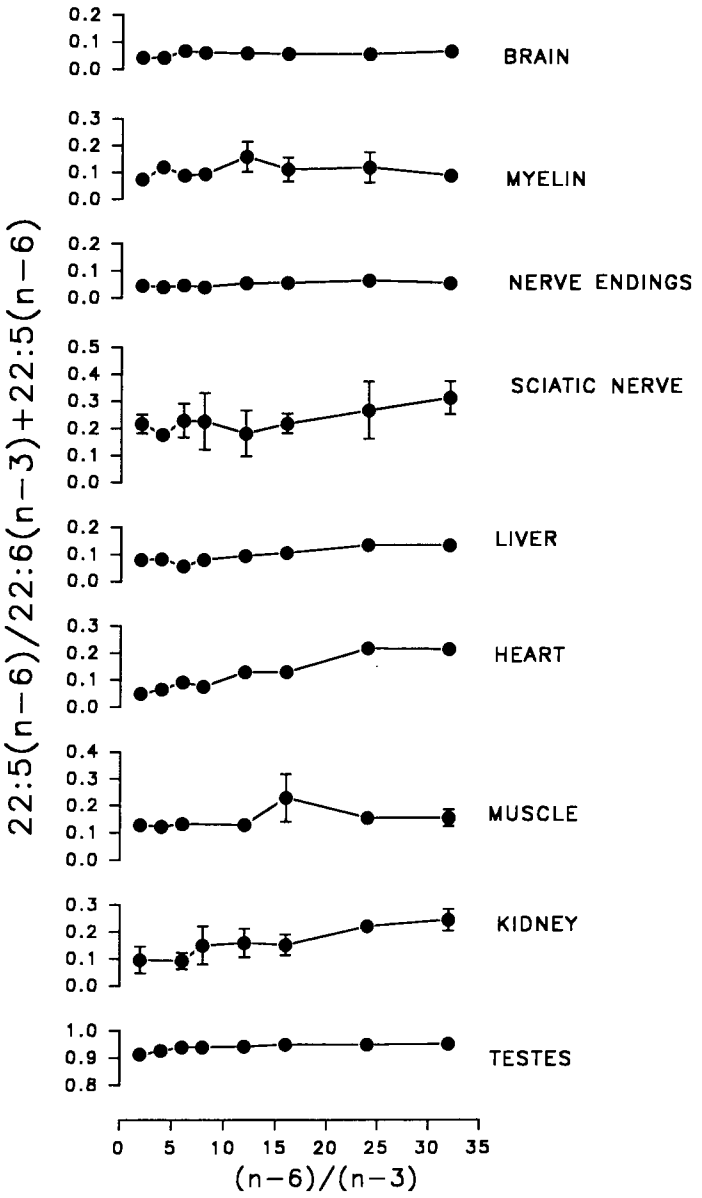

Figure 3. Ratio 22:5(n-6)/22:6(n-3)+22:5(n-6) as a function of dietary (n-6)/(n-3) ratio in various tissues.

(iii) The (n-6)/n-3) ratio and the amount of linoleic acid do not seem to be very effective parameters for measuring the effect of high levels of linoleic acid on fatty acids of the (n-3) series in different tissues.

It should be noted that in previous experiments performed under the same conditions but in which the level of alpha-linolenic acid varied (and not linoleic acid as here), it was possible to determine the minimum quantity of alpha-linolenic acid required to maintain docosahexaenoic acid levels in the different tissues and subcellular fractions (19). The amount of alpha-linolenic acid in the diet thus seems to constitute a more important limiting factor than that of linoleic acid. Whatever the case, the (n-6)/n-3) ratio and dietary linoleic acid content have little (heart and muscle) or no (brain, sciatic nerve, liver, kidney) effect on the 22:6(n-3)/22:6(n-3) + 22:5(n-6) ratio. On the other hand, the effect is considerable in testes, but these organs have an unusual polyunsaturated fatty acid metabolism.

Curiously few experiments have been directed at measuring specifically the effects of increasing levels of linoleic acid in the diet. In rodents, it has been previously shown that the liver stores arachidonic acid, which is afterward supplied to the other organs (20,21). Interestingly, when linoleic acid was increased to 10-11 %, arachidonic acid in various extrahepatic cells was reduced (22,25). Thus, according to Marangoni (1) in extrahepatic cells, remodelling of phospholipids through fatty acid replacement seems to prevail over the metabolic conversion of short-chain to long-chain polyunsaturated fatty acids when linoleic acid is increased in the diet.

When linoleic acid in the diet was raised from 2 to 10% of the energy intake, linoleic acid, arachidonic acid and n-3 fatty acids in plasma, liver and organs such as heart and kidneys were affected to different degrees (1). In all tissues, increasing dietary linoleic acid resulted in a parallel increase in levels of this acid. In contrast, elevation of arachidonic acid occurred only in the liver, with reduction in the heart and practically no change in plasma and in the kidneys.

In general, modifications of arachidonic acid levels in heart lipids have also been observed in animals fed different types of fats (26 ; 27) but these studies limited their comparisons to only a few scattered levels of dietary linoleic acid, and observed the reduction of long chain polyunsaturated fatty acids in the heart only at very high amounts of dietary linoleic acid.

According to Marangoni (1) the accumulation of linoleic acid differentially affects the levels of n-3 fatty acids in the liver and other organs. In the liver, there was no appreciable change in EPA and DHA levels, suggesting that the elevation of n-6 fatty acids does not affect the n-3 fatty acid pool.

Interestingly, the sum of (n-3) and (n-6) polyunsaturated fatty acids decreases in the liver (but not in other organs) when the dietary (n-6)/n-3) ratio increases (Figure 4).
For 150 mg alpha-linolenic acid per 100 g diet, the minimum amount of linoleic acid required is 1200 mg/100 g, as indicated by results in liver. In view of results in liver and brain, the optimal ratio is about 8. If the (n-6)/n-3) ratio is increased while the alpha-linolenic acid content remains the same, there is no alteration in cervonic acid concentration in brain, kidney, liver, sciatic nerve, or muscle; on the other hand, there is a slight linear decrease in heart and a considerable decrease in testes. The physiological and functional significance of these variations in the last two organs remains to be determined.

During the gestational-lactating period, it was surprising to find that low levels of dietary linoleic acid (0.3% of calories, 150 mg/100 g. food intake) did not have any effect on reproduction, gestation, perinatal mortality, body weight increase, or overall mortality. In fact, as long as the minimal quantity of alpha-linolenic acid (200 mg/100 g) was supplied, a linoleic acid intake of 150 mg/100 g covered the minimal requirements of certain organs and of certain fatty acids indicating that the level of arachidonic acid was constant and did not increase with incraesed dietary linoleic acid. However, the minimal requirement of the organ most dependent on linoleic acid (the liver) was 1200 mg/ 100 g food intake (2.4% of calories).

The level of the n-6 series polyunsaturated fatty acids in the brain varies with dietary linoleic acid content (28). The quantity of 2.4% of calories is higher than that previously proposed (29) for male (1.3% of calories) and female rats (0.5% of calories), but is the level generally accepted in human nutrition (30,32). In the adult male, 1.3% (33), and in children 3% (34) of calories are considered sufficient. In pigs the figure is 0.7% (35).
Interestingly, determination of the effect of dietary arachidonic acid in liver and in peritoneal exudate cell suggests that the type of (n-6) fatty acid (linoleic or arachidonic) could be important, as they may play a role in tissue composition (36). Consequently, the exact role of arachidonic acid in the diet remains to be elucidated.
Brain linoleic acid requirements are very high in man during the perinatal period (37,38). However, it should be noted that high concentrations of linoleic acid during total parenteral nutrition in newborns alter the fatty acid profiles of liver and brain (39). Thus, the effect of very high amounts of linoleic acid in the diet in adult animals remains to be elucidated. But such experiments depend on the chemical synthesis of triglycerides formed with linoleic acid alone (the vegetable oils always contain other fatty acids besides linoleic acid). It should be noted that direct uptake of

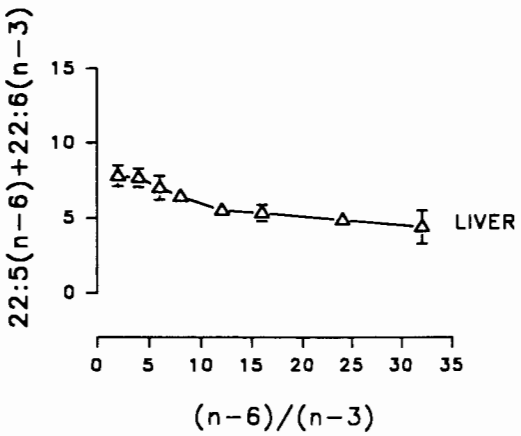

Figure 4. Sum of (n-6) and (n-3) fatty acids in liver as a function of dietary (n-6)/(n-3) ratio.

polyunsaturated fatty acids from the blood (associated with a low turnover) may be fundamentally involved in maintaining the fatty acid pattern of brain membrane lipids (40).

In conclusion, this work has shown that when the (n-6)/n-3) ratio in the diet varies from 2 to 32, and with a constant intake of 150 mg alpha-linolenic acid per 100 g diet, there is no change in the (n-3) series fatty acids, except for heart and testes. Results for the (n-6) series are more variable, especially for 22:5(n-6). The optimal (n-6)/n-3) ratio that should be provided in the diet is about 6. This experiment permitted determination of the minimum quantity of dietary linoleic acid needed to maintain linoleic family fatty acids levels in the organ with the highest requirement (liver) in the young adult rat: 1200 mg/100 g diet for liver.

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